Volume I.] *January, 1900.* [*No. 2.*

BIOLOGICAL BULLETIN.

THE EARLY STAGES IN THE DEVELOPMENT OF THE HYPOPHYSIS OF AMIA CALVA.

J. M. PRATHER.[1]

THE results of my work on Amia do not agree with the common assumption that the pituitary body is always of epiblastic origin. This disagreement has led me to an examination of the literature on the subject to see if there is sufficient unity of opinion among recent investigators to warrant such a general conclusion. As a result it is found that a diversity of opinion still prevails, and that it is unsafe to predict its origin in any class of vertebrates.

A brief classification of the various views and their respective advocates is of interest in this connection: K. E. von Baer ('**28**), Huschke ('**54**), and F. Schmidt ('**62**) believed the hypophysis to be a modified part of the brain. Reichert ('**40**) and His ('**68**) claimed that it is derived from the end of the chorda. Reichert ('**61**) and Rathke ('**61**) believed it to be derived from the pia mater, each having changed his earlier view. Dursy ('**68**) maintained that it is of threefold origin — from the fore-gut, the chorda, and the brain.

The above represent the earlier but now generally discarded hypotheses. The more modern views, some of which were also held by the older anatomists, may be grouped as follows:

1. *That the hypophysis is of hypoblastic origin,* as held by

[1] This study was undertaken at the suggestion of Dr. A. C. Eycleshymer and completed under his direction in the Department of Anatomy and Histology in the University of Chicago during the summer of 1899.

Rathke ('**38**), Luschke ('**60**), Kölliker ('**61**), Miklucho-Maclay ('**70**), W. Müller ('**71**), His ('**75**), Hatschek ('**81**), Dohrn ('**81**), Owen ('**82**), Balfour and Parker ('**82**).

It should be emphasized that, in general, this was claimed for the particular form investigated, but not claimed to hold true for all vertebrates.

2. *That the hypophysis is of epiblastic origin*, as claimed by Goette ('**72**), Balfour ('**74**), Mihalkovics ('**74**), Kölliker ('**76**), Cattie ('**81**), Julin ('**81**), Dohrn ('**82**), Kraushaar ('**83**), Johnson and Sheldon ('**86**), Orr ('**87**), Scott ('**87**), Kupffer ('**90**), Lundborg ('**94**), Dean ('**96**), Haller ('**96**), Braem (**98**), Minot ('**98**).

It should be added that the majority of these observers not only claimed the hypophysis to be of epiblastic origin in the particular form examined, but also believed this to hold good for the Vertebrata.

3. *That the hypophysis is partially of hypoblastic and partially of epiblastic origin*, as positively maintained by Kupffer ('**93**), Valenti ('**95**), and Nussbaum ('**96**), and considered probable by Hoffmann ('**85**), Orr ('**87**), and Gaupp ('**93**).

The researches made by the above-named writers show that the organ varies so much in its development and structure in the different forms that generalizations should be made with extreme caution, until more extended and precise observations have been made.

While its development has been more or less carefully traced in animals representing nearly every group of vertebrates, I find that the Ganoids have received but little attention. Kupffer has described and figured its earliest stages in *Acipenser sturio*. This author finds such an unusual mode of development that it seems possible that he has misinterpreted certain structures connected with the development of the sucking discs, for, as the sequel will show, at a certain stage in their formation in Amia these discs present appearances very similar to those regarded by him as the beginnings of the hypophysis. A fuller discussion is reserved for a later page.

Balfour and Parker have figured some of its early stages in *Lepidosteus osseus*, but have given no adequate description of its origin. Judging from their figures and few

remarks, its early history is in most respects quite similar to that in Amia.

Dean has figured a very early and a very late stage in its formation in *Amia calva.* No description is given of these stages, but the generalizations made therefrom seem, in the light of my researches, to be somewhat questionable, and to them I shall later recur.

Concerning the observations by Professor Minot I know nothing further than the simple statement in *Science*, Feb. 18, 1898, that he had confirmed and extended the results of B. Haller. As Haller's extensive observations did not embrace the Ganoids, I infer that this statement does not pertain to Minot's investigations upon Amia.

With these facts before us it will be seen that a detailed account of the development of the hypophysis in the last mentioned form will not be superfluous.

The sections for this study were placed at my disposal by Dr. Eycleshymer, and consist of series of sagittal, horizontal, and transverse sections of the embryo and larva, in many successive stages of development, up to and including the thirty-fifth day after fertilization. All ages given in the following descriptions are reckoned from the time of fertilization.

Many examinations were made with the oil-immersion and all the drawings have been made with the aid of the camera lucida. The literature consulted, and that to which reference has been made, consists for the most part of papers enumerated by Kupffer [1] and Haller.[2] Since a very complete bibliography is given by each of these authors it would be superfluous to duplicate them in the present paper.

Description of Stages.

The earliest stage in the formation of the hypophysis, clearly recognizable as such, is found in an embryo a few hours before

[1] Kupffer, C. von, "Die Entwicklung des Kopfes von Acipenser Sturio an Medianschnitten untersucht." München und Leipzig. 1893. "Die Deutung des Hirnanhanges," *Sitzungsber. d. Gesell. f. Morph. u. Phys. in München.* Jahrg. 1894.

[2] Haller, B., "Untersuchungen über die Hypophyse und die Infundibularorgane," *Morph. Jahrb.* 1897.

hatching, as shown in Figs. 2 and 3. In order to show the relations of position which the various organs bear to one another prior to the differentiation of the hypophysis, I figure a median sagittal section of an embryo surrounding about 245° of the circumference of the yolk, corresponding to an age of about 148 hours (Fig. 1). At this stage the foregut (*fg.*) is seen to have formed as far back as the posterior limit of the third primary vesicle (*hb.*), where the endoderm forming its wall is reflexed upon itself and passes forwards again over the surface of the yolk (*y.*). By tracing the foregut through the successive sections of the series, we find it to be a very broad cavity compressed dorso-ventrally until its upper and lower walls are in close apposition in the region immediately under the base of the first primary vesicle, which rests directly upon its dorsal wall. Its walls are slightly separated both anterior and posterior to this region. In the median plane, as shown by the figure, the cavity can be traced forwards to a point somewhat anterior to the front wall of the brain (*br.*). From its anterior end a diverticulum may be traced on either side in front of the brain, nearly to the dorsal median plane. These endodermal diverticula later become transformed into the larval adhesive organ, as has recently been shown. The endodermic layer increases in thickness anteriorly until a maximum thickness is attained in the adhesive organ just mentioned. The walls of the endoderm cells cannot be distinguished, owing to the great amount of yolk material found in them.

The ectoderm at the anterior end is invaginated in two places. The upper invagination (*o.*) is merely a depression within the adhesive organ which is developing beneath the ectoderm by diverticula from the foregut, as just described, pushing the ectoderm outwards in the form of a circular ridge (*ao.*) around the snout. The lower invagination is the involution for the stomodaeum (*st.*), and has already pushed inwards nearly to the endoderm surrounding the foregut. This invagination is on a lower plane than the foregut and is directed distinctly downwards, making a small angle with that plane.

The large cavity (*c.*) between the brain and the epiblast in front is for the most part filled by the developing adhesive

organ. But surrounding this may be seen mesodermal cells which form a strand running downwards between the stomodaeum and foregut and connecting with those forming the heart (*h.*) below. The chorda extends no further forwards than the posterior margin of the hind brain. The brain is not as clearly delimited from the ectoderm above as the figure indicates. The first primary vesicle (*fv.*) is evaginated at its base both in front and behind, giving rise to the recessus opticus (*ro.*) and the infundibulum (*in.*) respectively. The base of this vesicle is arched considerably and conforms closely to the dorsal wall of the foregut.

In an embryo about 160 hours old (Fig. 2) marked changes may be noted in all the organs described in the preceding stage. The adhesive organ has broken through the ectoderm, forming a semicircular row of sucking cups on either side of the snout. The section, not being exactly vertical, passes through one of these cups (*sc.*) on the dorsal side. The space between this organ and the brain is now filled with mesoblastic cells (*ms.*). The stomodaeal invagination has deepened a little, owing to the development of the sucking disc over and in front of it. By the vertical growth of the brain the anterior end of the alimentary canal has been pushed downwards to a level with the mouth fold, so that the ectoderm lining the stomodaeum is in close contact with the endoderm forming the wall of the foregut. The oral plate (*op.*) thus formed is on the point of breaking through, and the point of fusion of the endoderm roofing the gut with the ectodermal roof of the stomodaeum is scarcely recognizable. There is no fold of the ectoderm comparable to "Rathke's pocket" discernible, nor is there an endodermal fold comparable to "Seesel's pocket." The ectoderm immediately over the stomodaeum is much thickened and composed of large cells of irregular shape loosely aggregated.

Fig. 3, an enlarged portion of the same section, will show that this ectodermal layer (*ec.*) terminates rather abruptly at the point of junction (*pf.*) with the endoderm (*en.*), at which point the cells are seen suddenly to become smaller. While they are somewhat disconnected in this region, they soon become arranged into three definite layers running back under the ante-

rior part of the thalamencephalon as far as the posterior limit of the optic chiasma (*oc.*). Here, immediately under the central part of the thalamencephalon (*lpo.*), the hypoblastic cells may be seen to have assumed a different shape and size, and the number of layers to have increased. From roundish or ovoid cells they become long, spindle-shaped, much smaller, and arranged in crescent-shaped layers about six in number and fitting one within another, so that the whole mass of cells over a region about 180 μ in length is nested in this peculiar manner. This is the first trace of the hypophysis (*hy.*) recognizable. Back of this differentiation the cells of the endoderm are again of the same rounded or ovoid shape as in front and arranged in two layers. Thus, I repeat, there can be detected no fold or overgrowth of ectoderm to give rise to the hypophysis nor evagination of the endoderm. Its cells are differentiated *in situ* apparently by longitudinal division of the cells constituting the roof of the foregut.

The chorda at this stage extends forwards under the hind brain to near the tip of the infundibular fold, which has greatly enlarged. The base of the thalamencephalon has elongated and is now a single layer of cells (*lpo.*) posterior to the chiasma, resting closely upon the dorsal wall of the foregut.

In a larva early in the eighth day, shortly after hatching, a sagittal section (Fig. 4) shows the oral plate broken in the center. But sections of the series on either side show the membrane or its remnants still intact, stretching across the oral cavity from a point near the tip of the now forming lower jaw to a point (*op.*) on the dorsal side of the cavity just forwards of the anterior limit of the thalamencephalon where it rests upon the dorsal wall of the foregut. It is thus seen that the position of the membrane relative to surrounding parts has not changed from the preceding stage. The floor of the foregut, however, has dropped down in the anterior part, making a rather deep cavity (*fg.*) immediately below the thalamencephalon and behind the oral plate. The dimensions of the thalamencephalon have increased vertically and decreased antero-posteriorly. The infundibular fold has apparently widened, but not deepened. The optic chiasma has greatly thickened, while the base of the

brain remains one cell in thickness and rests flatly upon the dorsal wall of the gut and the hypophysis. The sucking disc (*sc.*) is relatively at its largest size. The cavity between it and the brain is filled with a denser aggregation of mesoblastic cells than in the previous stage.

Fig. 5 shows the base of the brain and the hypophysis of the same section more highly magnified. The hypophysis now measures 150 μ in length and 28 μ in thickness. This apparent shortening may be due to the uncertainty of the limits of the organ in the previous stage, or to individual variations in the two larvae. That peculiar nesting of the cells in the hypophysis, as described above, will be seen to continue, but the cells are now perceptibly larger, while the epithelial cells (*em.*) forming the roof of the mouth beneath the hypophysis have again attained the size and shape of those with which they are continuous in front and behind. The basal layers of endoderm, however, still seem to be dividing, adding new layers to the base of the hypophysis by proliferation. The roof of the mouth is now three layers deep both before and behind the hypophysis.

A transverse section (Fig. 6) of a larva about eight days old, through the posterior part of the hypophysis and the infundibulum, shows that the organ is at this stage convex on the dorsal side in a transverse plane, fitting into a concavity in the base of the infundibulum lying closely upon it. An examination of the successive sections forwards and backwards from this shows that the upward convexity gradually diminishes forwards, but more abruptly backwards. This figure, in connection with the figures of longitudinal sections of larvae both older and younger, shows that the organ is now approximately lenticular in shape. In transverse, as in longitudinal sections, that characteristic nesting of the cells differentiated to form the hypophysis is found to prevail, a basin-shaped stratum of lenticular cells formed from the deeper layers of the endoderm, with other similar strata, each successively smaller, fitting into the previous ones, until we get a nest of seven or eight basins, while the hollow of the upper basin is filled to a rounded fullness with cells of a more nearly uniform diameter, rounded or

polygonal, and with no evident order of arrangement. This fact is strikingly evident, that the limits of the organ, both longitudinally and transversely, mark very accurately the borders of the area over which the brain is in such close contact with the hypoblast.

The mouth cavity posterior to the oral plate is now rather shallow but very wide. Just over the oral epithelium roofing the mouth, on either side of the hypophysis and the infundibulum, the internal carotid arteries (*v.*) are forming, while dorsal to these, in the folds formed between the infundibular lobe and the thalamocoele above, may be seen two other large cavities (v'), the cavities of the preoral somites.

A sagittal section of a larva about nine days old (Fig. 7) shows the base of the thalamencephalon (*fv.*) to be a single layer of columnar cells where it rests on the hypophysis, but thickened in front for the chiasma and likewise behind in the infundibular region, which has grown larger. The end of the chorda approaches very near to the infundibulum, but remains separated therefrom by mesoblastic cells, which may be seen to grow in between the brain and mouth roof to the limits of the hypophysis at either end. The lower jaw is relatively further back, its tip now lying under the anterior end of the hypophysis. The epithelium of the mouth is continuous beneath the hypophysis, but the basal layer of endoderm appears to continue to divide, adding new cells to the hypophysis by proliferation. The marked difference between the shape and arrangement of the cells in the upper and lower portions of the organ may be noticed still. At the posterior end the body is distinctly separated from the mother layer, while at the anterior end its cells take on more and more the character of the mother layer as we go forwards. The organ measures in this plane 196 μ in length by 41 μ in thickness.

A transverse section of a slightly older stage (Fig. 8), during the tenth day, shows an oval-shaped organ 130 μ in breadth by 64 μ in thickness. It is clearly separated from the endoderm at either side, so that it seems to be wholly differentiated, but still lying in a depression in the endoderm caused by the transformation of the cells of this layer into cells of the hypophysis.

The characteristic stratification on the ventral side and the irregular grouping of the cells above are still pronounced. Very nearly in the center, between these lower strata and the cells above, is a nearly spherical cavity (*l.*) about 16 μ in diameter, the first lumen that has been recognized. This lumen is not a longitudinal slit, as it is found only in this section; and since it is enveloped by no distinct membrane, and the cells have no definite arrangement about it, it has the appearance of an intercellular space. The mesoderm (*ms.*) is seen to grow between the brain and the mouth up to the sides of the hypophysis, just as at the ends. It would thus appear to be a biconvex body fitting into concavities in the brain above and the endoderm below.

In a larva ten days old, of which Fig. 9 is a sagittal section of the hypophysis and parts adjacent and Fig. 10 a transverse section more highly magnified, the organ has become entirely separated from the mouth roof, which has again become two cells in thickness beneath it. A strand of mesoderm (*ms.*) passes between the hypophysis and mouth roof and is continuous at the sides with the thickened mesoderm differentiating to form the cranial cartilages. An outfold of the infundibular wall at its posterior lower margin, 33 μ by 39 μ internal measurement, is the first stage in the formation of the infundibular gland, or saccus vasculosus (*sv.*). The single layer of cells characterizing the base of the infundibulum and the base of the thalamencephalon is continued into the saccus, but here the cells are columnar, with nuclei at their outer ends, in marked contrast with the cells in the brain wall contiguous. The chorda (*ch.*) runs forwards under the hind brain almost in contact with the roof of the mouth, and its cephalic end (*chh.*) abuts against this infundibular process with a strand of mesoderm between. This mesoderm grows into the space about the hypophysis, and a thin strand of it runs between the organ and the mouth roof below.

The hypophysis now measures in longitudinal section 143 μ by 57 μ, in transverse 136 μ by 66 μ. A lumen may be seen in the center, — an oval cavity with a membrane surrounding. About this lumen the spindle-shaped cells seem to be arranged in a radiate fashion, with long axes pointing towards it.

In a larva about fourteen days old a median sagittal section (Fig. 11) shows marked changes in surrounding parts, with but little change in the hypophysis. The saccus vasculosus has grown until it measures at this stage 120 μ in length, and from 6 μ in width at its point of origin to 27 μ at its widest part. Its tip now touches the base of the hind brain. The columnar cells which characterized it in the preceding stage are yet very noticeable, the transition to the rounder cells of the infundibular wall above being very abrupt, while the transition to the cells of the infundibular base is more gradual. Beneath, the hypophysis rests directly upon a strand of fibrous tissue (*ms.*) continuous before and behind with the perichondrium surrounding the sphenoidal cartilages, which are encroaching from all sides. The membrane roofing the mouth (*em.*) has become widely separated from the hypophysis, and in it dental protuberances and glandular cells have differentiated. The hypophysis has now attained a size of 156 μ by 56 μ, and has several small spherical or lenticular cavities which do not communicate.

A sagittal section of the hypophysis of a larva about the same age is shown in Fig. 12 strongly magnified. It will be seen that a few mesodermal cells have come to lie between the hypophysis and the brain, forming a thin layer separating the two organs. With this interpolation of mesoblastic tissue the lobing of the hypophysis begins, and this continues to increase with age. A large central oval lumen is conspicuous at every stage, while in the section here figured, several smaller lumina are met with as one examines the sections of the series. The principal lumen in this case measures 19 μ by 6 μ, and can be detected in but two sections, showing that its shape is lenticular. The smaller ones are more nearly spherical, and each is found in but a single section. No communication between them can be found. The hypophysis in this larva measures 164 μ in length by 57 μ in thickness. The characteristic radiate arrangement of the cells about the lumina is very noticeable.

Fig. 13 shows the relations of the hypophysis to surrounding parts, at a stage about one day older, in a transverse section through the middle of the organ. The oral epithelium has

further differentiated. The lateral cartilages (*sk.*) of the skull are closely encroaching from the sides, enclosing the internal carotids (*bv*). These cartilages are connected by a strand of the perichondrial membrane which runs beneath the hypophysis. The organ is almost perfectly lenticular in cross-section, measuring 180 μ by 70 μ. A principal lumen is seen in the center, but others are found at other positions in sections in front and back of this one.

In a larva between twenty-two and twenty-six days old, a cross-section (Fig. 14) shows the hypophysis appreciably enlarged, measuring now 220 μ by 75 μ. An irregular but distinct lobing may be seen, more pronounced on the upper than on the lower side. In this way, from a very symmetrical organ in the fifteen-days stage, we get here a marked asymmetry. It is here firmly adherent to the infundibulum, but quite apart from the wall of the cranial cavity. This separation from the base of the cranial cavity may be considered an artificial condition, as succeeding stages invariably show it to be in contact with this wall. The distinct upward bend in the infundibular base, the enlargement and gradual encroachment of the lateral cartilages, and the advancing differentiation of organs in the oral epithelium may be remarked.

The same section of the hypophysis highly magnified (Fig. 15) shows its histological structure to present some interesting features. The large central lumen with its very definite lining membrane is a striking object. It is ovoid, measuring 24 μ by 14 μ. Other spheroidal but smaller lumina are to be found near the tip of the different lobes as they are traced by sections, but communication of these lumina with each other, or with the central lumen, cannot be definitely proved. It seems that communication may be had through very narrow channels representing connected intercellular spaces between rather definite rows of cells and running from the lumina of the lobes towards the central lumen. But apparently these channels are closed before reaching the lumen. Such spaces may be seen in the figure (*sl.*) running out into the lobes to the right and to the left. It will be noticed that the cells maintain a rather definite and orderly arrangement about the central lumen. There is a

row encircling the lumen in a radiate manner, long spindle-shaped in the upper and lateral parts, more rounded below. The other cells appear to have a general tendency to arrange themselves in rows pointing towards the center of the organ, but this arrangement is modified by a secondary tendency to be grouped about the secondary lumina and the longitudinal channels. A marked feature of this and succeeding stages is the indistinctness or total obscurity of the nuclei.

A sagittal section at this stage shows the same peculiar arrangement of cells about the lumina and channels in the separate lobes. And the other characters of the hypophysis are similar to those shown in the cross-section. The anterior sphenoidal cartilage has now advanced to the posterior part of the chiasma nearing the hypophysis, the posterior to a point not far from the posterior point of the saccus, while dense skeletogenous tissue continues to and below the saccus nearly to the hypophysis. The perichondrium, as before, stretches across from one cartilage to the other below the organ. The basilar artery is now well formed, running along the base of the hind brain up into the fold between the hind brain and the primary forebrain. A blood vessel may be seen also just posterior to the hypophysis beneath the point of origin of the saccus. The saccus has enlarged, and in its cavity may be seen abundant granular secretions.

Passing from this stage to a stage between thirty and thirty-five days, a sagittal section (Fig. 16) shows all parts much enlarged. The finger-shaped saccus measures internally 311 μ by 18 μ at its narrow opening into the infundibulum, and 77 μ at its widest part. The granular secretions noticed in the previous stage have increased in amount. While the sphenoidal cartilages have enlarged and strengthened compared with the condition in the previous stage, they have approached very little nearer to the hypophysis; but the connective tissue between the hypophysis and the roof of the mouth has considerably increased. The hypophysis at this stage has attained a size of 359 μ by 96 μ. Increased lobing is not apparent from the figure, but the series shows a great increase in the number of lobes and of the lumina in them. The arrangement and char-

acteristics of the cells are not enough different from those described in the preceding stage to call for special remark.

A comparison of sagittal with transverse sections (Fig. 17) demonstrates that in shape the organ retains the general lenticular form acquired early in its formation. It measures now $284\,\mu$ in breadth by $88\,\mu$ in thickness. The lateral cartilages (*sk.*) have advanced far in towards the hypophysis, so that it may be clearly seen that the organ lies in a distinctive space surrounded by cartilages on all sides — the pituitary fossa. Small bits of connective tissue may be seen between the hypophysis and the brain in the folds of the former, and also in the recesses between the lobes on the under side. The infundibular base is folded more or less in conformity to the lobing of the hypophysis. No evident communication between the cavities nor ducts opening to the exterior have been observed at this, the latest stage studied.

A horizontal section of the hypophysis (Fig. 18) of a larva about 20 mm. in length, thirty days old, shows that the lobing of the organ is principally around the edge ; that its general shape is circular in this plane, lenticular as a solid ; that a cavity may be found near the end of each well-formed lobe, which may possibly communicate with the central lumen by a very narrow indistinctly defined channel ; that the cells are, in general, arranged in a double row around each lobe, the space between the rows constituting the channel mentioned ; that the cells are arranged radially about the lumina. The section figured is not exactly in a horizontal plane, but dips a little posteriorly and to the right, so that the lobes mostly appear to be on the anterior side, but are in reality of approximately the same number in each half. The organ is here seen to be enclosed in the sella turcica, which is far advanced in its formation. The infundibulum fits closely upon its upper surface, the projections of the one fitting roughly into the depressions of the other. No blood vessels can at this stage be seen entering the organ, nor nervous tissue be found connecting it with the brain. It seems not to have become glandular as yet.

SUMMARY.

In distinction from an epiblastic origin, as found in most forms, my observations lead me to believe that the hypophysis is of hypoblastic origin in Amia.

Prior to the differentiation of the hypophysis the foregut extends far forwards ; by diverticula the hypoblast reaches even the dorsal side of the head in front of the brain. At this time the stomodaeal involution is below the front end of the foregut. The diverticula later sever their connection with the foregut and are metamorphosed into the larval adhesive organ. The hypoblast unites with the epiblast on the last day before hatching, seventh day, forming the oral plate at a point forwards of the anterior limit of the brain.

There is no indication of an overgrowth of epiblast between the brain and the foregut, nor is there an invagination from the stomodaeum to give rise to the pituitary body. Neither does an outfold from the hypoblast occur to form it. Its first stage is found near the close of the embryonic period, about 160 hours, as a local differentiation of hypoblastic cells in the dorsal wall of the mesenteron, immediately under the thalamencephalon where the base of the brain is in close contact with the hypoblast. The intimate fusion of the base of the first primary vesicle with the hypoblast, from a time long before the stomodaeum has united with the foregut until after the hypophysis has become well differentiated, seems to me to preclude the possibility of any epiblastic tissue entering into its composition, and leads me to think that its origin is probably due to a mechanical cause. This region of fusion is far back of the oral plate, which remains intact for several hours after the differentiation of the hypophysis has begun.

The growth of the hypophysis is at first apparently due more to the enlargement of the cells first differentiated to form it than to the addition or multiplication of cells. It appears to remain in genetic connection with the mother layer until about the tenth day, when it becomes wholly delimited therefrom by an ingrowth of mesoblastic tissue between it and the mouth roof.

During this early period there is a distinct stratification in the arrangement of cells in its lower portion, as if formed in successive strata by proliferation from the mother layer. This stratification is not apparent in the upper part of the organ, and is no longer seen in the lower part after the ninth day.

The first lumen is formed about the ninth day, from which time on an increasing number of lumina is to be found as the organ develops. A lumen appears near the end of each lobe. These lumina are oval or spherical cavities, and definite channels of communication have not been observed, though indications that such channels are forming are found in the arrangement of the cells about the longitudinal axes of the lobes. The cells have a tendency to arrange themselves radially about the lumina.

The organ is almost perfectly lens-shaped until about the fifteenth day, when the formation of lobes begins with the interpolation of mesoblastic cells between the hypophysis and the brain. The number of lobes multiplies from this time on till the thirty-fifth day, beyond which stage observations have not been made. The lobes form chiefly around the periphery of the lenticular body.

The organ is thus, at this stage, a spongy body with isolated cavities, rather than a complex of glandular tubules which so frequently characterize it. The nuclei of the cells become indistinct or wholly disappear by the twenty-second day. This may indicate a glandular modification, but no evident glandular secretions have been detected. No duct nor external opening of cavities has been observed.

No arteries or blood vessels are to be found in it at the latest stage examined. Neither have nerve fibers been seen connecting it with the brain.

The cranial cartilages developing from the mesoblast increase in size and strength from about the tenth day, until at the thirty-fifth day they closely surround the organ on all sides, forming the pituitary fossa, in which the organ lies in close contact with the infundibulum on the dorsal side, but separated from the mouth by a fibrous strand of connective tissue on the ventral.

The saccus vasculosus begins to form about the tenth day and steadily enlarges, until at the thirty-fifth day it forms a process of the infundibulum in the shape of a glove-finger, extending directly backwards under the base of the medulla and parallel with the base of the cranium.

This is very nearly its position in the adult brain as figured by Allis.[1] At no stage, therefore, is it in close association with the hypophysis, which connection is found to be true in so many cases. Abundant granular secretions are found in it at the twenty-second-day stage, and these increase in amount thereafter.

General Remarks.

The course of development of the hypophysis in Amia as described in the preceding pages, when compared with the descriptions given by other observers, both in Amia and other Ganoids, will be seen to present some striking peculiarities.

Dean[2] says: "The hypophysis is by no means as important an element in the development of the head in Amia as in other Ganoids. Its appearance is late and inconspicuous. It has not been found in stages earlier than that of Fig. O (*hatching time*),[3] and even here its presence is not definite. At the most the position of its lumen can be recognized as the line *HY*, formed by the arrangement of cells immediately below the region of the recessus opticus. These cells are apparently ectodermal, for they are arranged in a continuous line with the cells of the formative epiblast of the dorsal wall of the stomodaeum, but, on the other hand, their ventral limit cannot be distinguished from the entodermal cells roofing the foregut."

In what respect the development of the hypophysis is less important in Amia than in other Ganoids is not clear. In point of size and position its relations are almost exactly the same as in the other Ganoids, so far as the larval stages have been examined. As for its appearance being "late and incon-

[1] Allis, Edward Phelps, "The Cranial Muscles and Cranial and First Spinal Nerves in Amia Calva," *Journ. of Morph.* Vol. xii, No. 3, Pl. XXXVIII. 1897.

[3] Italics are mine.

[2] "On the Larval Development of Amia Calva," *Zool. Jahrb.*, p. 667. 1896.

spicuous," I may say that it arises, as will be shown, at a time intermediate between the time of its first occurrence in the other two forms in which this point has been determined, and it is certainly a conspicuous, I might say, striking differentiation of cells, even in its fundaments, although it may not be a prominent object in point of size or in the distinctness with which its limits may be fixed. The actual time of its appearance I have found to be several hours earlier than the time assigned by Dr. Dean, as a comparison of Fig. 4 with his Fig. O plainly proves. His figure and description indicate a stage nearly the same as my Fig. 4, which is shortly after the time of hatching. Although in his figure the oral plate is still unbroken, the cavity of the foregut posterior to it has noticeably deepened by the dropping downwards of its basal wall, while in the stage I figure the cavity is of about the same depth, and the oral plate is only severed at its middle point, remaining intact at the sides. While in Fig. 4 the cells, which he would call the beginning of the hypophysis, may be said to be "continuous with the cells of the formative epiblast," being in the same plane with them, there is no other evidence of a connection and certainly no ground whatever for considering them derived therefrom, as the enlargement of this section (Fig. 5) makes very clear their differentiation from cells of the hypoblast. But its earliest stage is found (Figs. 2 and 3) some hours previous to this stage, far back of the oral plate and the epiblast, as a well-defined modification of cells of the basal layer of hypoblast where the post-optical lamina of the brain rests closely upon it. Considering the common assumption of embryologists that its origin is from the epiblast, my observations become of interest, since I believe that I have proved beyond question that the hypophysis is of hypoblastic origin, and I do not at present understand how Dean could have considered it otherwise.

Balfour and Parker[1] state in a footnote: "We have not been able to work out the early development of the hypophysis as satisfactorily as we could have wished. . . . Were it not for

[1] "On the Structure and Development of Lepidosteus," *Trans. Roy. Soc.* Pt. ii, p. 379. 1882.

the evidence in other types of its being derived from the epiblast, we should be inclined to regard it as hypoblastic in origin." They speak of it as an invagination of the oral epithelium, without stating whether this invagination is anterior or posterior to the oral plate. Presumably from the previous statement they consider it derived from the roof of the foregut posterior to the oral plate. They figure a transverse section of the anterior part of the head of an embryo on the ninth day after impregnation, showing an invagination from the mouth roof with a thickened, solid, conical process extending upwards into the cranial cavity. This, with the exception of the invagination, is very similar to its condition in cross-sections of Amia late in the seventh day, passing through the anterior end of the organ, as shown in Fig. 6. They also figure transverse sections through the anterior and posterior ends of the hypophysis of an embryo eleven days old, where in front it is still in connection with the oral epithelium, while behind it is constricted from that layer. These sections indicate relations almost exactly the same as transections of the organ in Amia shown in sagittal section (Fig. 7) at an age of not quite nine days. Comparing these few sections of Lepidosteus and the few words of description with the conditions which I find in Amia, it seems that the hypophysis undergoes a nearly parallel series of changes in the two forms from the time of its primary origin to a late larval stage, but that corresponding embryonic stages are met with in Amia from one and a half to three days earlier than in Lepidosteus, and the corresponding larval stages are found earlier and earlier as the animal advances in age. This justifies, so far as the hypophysis is concerned, Dean's statement that "the organogeny of Amia progresses more rapidly than in Lepidosteus."

I find no lumen earlier than the ninth day, when it appears as a single nearly spherical cavity near the center of the organ, rather than a longitudinal slit, as indicated by Dean in Fig. O. Furthermore, as my sections show, the lumen is never a longitudinal slit, even at the late stage of thirty-five days, corresponding to Dean's Fig. Q of a four-weeks-old larva which shows it as such. Instead of a single lumen at this late period,

I find several lumina which apparently do not communicate. Neither do my observations agree with those of Balfour and Parker on Lepidosteus at a corresponding age, where the hypophysis is described as "small, not divided into lobes, and provided with a very small lumen"; whereas in Amia at this period it is much lobed and possesses numerous spherical lumina.

Kupffer (*loc. cit.* p. 59) has described a very early and unusual formation of the hypophysis in *Acipenser sturio*. According to him the organ arises during the second day after fertilization by an invagination of the basal layer of the ectoderm on the dorsal side of the head, in front of the brain, between the yet unclosed neuropore and the sucking disc. This invagination grows downwards and backwards until it comes in contact with the endoderm, and then, before the close of the second day (forty-five hours), by a rupture of this layer, it communicates with the alimentary canal, which communication persists for about twenty-four hours. But before the stomodaeum has formed a connection with the foregut, this union of the hypophysis with the latter has broken off (*i.e.*, by the sixty-fourth hour), and the lower blind end of the tube becomes swollen into a hollow bulb. This bulb gradually enlarges and migrates backwards to a position between the dorsal wall of the gut and the base of the thalamencephalon, while the remaining part of the tube, namely, the stalk, gradually atrophies and has wholly disappeared by the time the embryo is hatched (eighty-seven hours).

Haller has expressed a doubt as to Kupffer's interpretations. This doubt is based upon the indistinctness of the parts observed at this early time. From Kupffer's own words it would seem that the cells were greatly obscured by food-yolk. I give the passage quoted by Haller: "Die Entodermzellen sind noch mit Dotter überladen, die Zellen der Epidermis und des Hirnes schon fast dotterfrei, aber diejenigen Ektodermzellen, die in der Bildung der gleich zu besprechenden Organe (*Hypophysenanlage*, etc., Haller) eingehen, zeigen noch denselben Dottervorrath wie die Elemente des Entoderms und sind daher durch Färbungen von diesen nicht zu unterscheiden. Die Abgren-

zung der einzelnen Theile dieser Region gelang mir erst unter vergleichender Prüfung nahe auf einander folgender älterer Stadien."

I likewise believe that Kupffer has misinterpreted what he saw, but for an entirely different reason from that given by Haller, yet based upon the same passage.

Miss Phelps[1] has recently shown that the "larval adhesive organ" in Amia develops at about the time that Kupffer assigns to the formation of the hypophysis in Acipenser. The adhesive organ begins as a diverticulum from the endoderm anterior to the brain, which later becomes divided into a pair of diverticula whose connection with the endoderm becomes broken off after a time. Acipenser and Lepidosteus each possesses a sucking disc, or adhesive organ, similar to that in Amia and at a corresponding developmental period. It would therefore seem that they should justly be regarded as homologous organs, and, further, we should naturally expect them to have a similar ontogeny. Previous to the observations of Miss Phelps this organ has been assumed to be of ectodermic origin (Dean for Amia, Balfour and Parker for Lepidosteus, and Kupffer for Acipenser).

In my own studies on Amia, as described on a preceding page, I observed these diverticula connecting the foregut with lateral masses of cells lying on either side of the snout and between the anterior end of the brain and the overlying epidermis, filling nearly the whole of this pre-cerebral region. These masses of cells present an appearance exactly like the cells walling the foregut, being laden with much yolk just as Kupffer describes, and could not be distinguished from them. The narrow channels can be traced from the cavity of the foregut upwards through the masses to near the epidermis on the dorsal side where they end blindly. The cells of the epidermis and brain contiguous to these present a very different appearance, due to their freedom from yolk.

I was at a loss to account for these canals connecting the enteric cavity with organs on the dorsal side of the head, until

[1] "On the Development of the Larval Adhesive Organ in Amia." Abstract in *Science*. March 10, 1899.

I saw the account of the development of the adhesive organ by Miss Phelps. I at once concluded that I had not only confirmed her discovery, but had also found wherein Kupffer has probably erred in his interpretation of the structures which he believed to be connected with the development of the hypophysis, but which to my mind have an entirely different meaning. The statement by Kupffer, that the cells overladen with yolk going to form the hypophysis are not to be distinguished from the elements of the endoderm, receives its explanation in the fact that they are endodermal cells growing out from the foregut by evagination. The canal which, according to Kupffer, connects the foregut with the dorsal ectoderm is nothing else, in the opinion of the writer, than this median diverticulum from the foregut, or possibly one of the lateral diverticula resulting from it, running up through the adhesive organ to the point of closure of the neuropore. A slightly oblique dorso-ventral section through this region in Amia during the sixth day of development would show strikingly similar appearances to those figured and described by Kupffer in Acipenser.

As a phylogenetic interpretation of the singular development of the hypophysis in Acipenser, Kupffer holds that we here have traces of the ancestral mouth which opened above the sucking disc and in front of the brain. Its origin from the stomodaeal roof as found in most vertebrates, he claims, is a secondary condition due to a migration downwards from its original position, compelled by the great development of the fore brain in these forms and the concomitant degeneration of the adhesive discs, whose remnants, he thinks, are still to be found in the fold between "Rathke's pocket" and the oral plate.

This explanation fails to account for its origin in Amia and Lepidosteus, in which the sucking disc is still large and functional, and whose fore brain is little different from that found in Acipenser; and yet in them the hypophysis arises at a point far back of the sucking disc and under the brain.

It may be considered highly probable that the hypophysis is

endodermic in origin in Lepidosteus as well as in Amia, and I venture the prediction that a further study of Acipenser will demonstrate the same for that form. The foregut extends far forwards in each of the Ganoids in which its development has been studied, and the writer believes that in all these it arises from the endoderm. A study of the figures of the head parts in those animals in which the hypophysis is undoubtedly of ectodermal origin shows that in them the foregut stops short of the infundibulum, while in some, at least, of those forms in which its origin is questionable — ectodermic or endodermic — the foregut and stomodaeum meet at an intermediate point, directly under the base of the thalamencephalon. These facts lead me to suggest that mechanical factors, acting at the point of fusion of the brain base to the oral roof, may play an important part in determining its development from this or that layer.

If my observations be confirmed, that the hypophysis in Amia is derived from the hypoblast, will this fact strengthen Kupffer's hypothesis that it represents a degenerated "paleostome," or will it rather revive the old theory of Dohrn, that it represents a portion of a canal connecting the alimentary tract with an exterior dorsal opening through the thalamencephalon and the epiphysis and accordingly believed to be homologous with the invertebrate pharynx? I find nothing in its structure or its relations, other than its point of origin, which can be interpreted as evidence in support of either hypothesis. I have given what I believe to be the facts, but must leave their interpretation to the maturer judgment that will come from the more complete and extended observations of the future.

HULL ANATOMICAL LABORATORY,
September 22, 1899.

ABBREVIATIONS.

A. anterior.
ao. outfold of ectoderm caused by the developing adhesive organ.
br. wall of brain.
bv. blood vessels.
c. cavity occupied by adhesive organ in its young stages.
ch. chorda.
chh. cephalic end of the chorda.
ec. ectoderm.
egm. egg membrane.
em. epithelium of mouth.
en. endoderm.
ep. epiphysis.
fg. foregut.
fv. first primary vesicle of brain.
h. position of heart.
hb. third primary vesicle of brain.
hy. hypophysis.
in. infundibulum.
l. lumen of hypophysis.
lpo. lamina postoptica.
ls. crystalline lens.
m. mouth cavity.
mb. mesencephalon.
ms. mesoderm.
o. involution of ectoderm between the sucking discs.
oc. optic chiasma.
op. oral plate.
ov. optic vesicle.
P. posterior.
pf. point of fusion of ectoderm with endoderm.
ro. recessus opticus.
s. strand of fibrous tissue continuous with the perichondrium.
sc. sucking cup.
sk. skeletal cartilages.
sl. cleft between rows of cells.
st. stomodaeum.
sv. saccus vasculosus or infundibular gland.
v. internal carotid artery.
v'. head cavities.
y. yolk.

EXPLANATION OF FIGURES.

FIG. 1. Median sagittal section of the anterior portion of an embryo surrounding about 245° of the egg's circumference. About 148 hours. × 60.

FIG. 2. Median sagittal section of the anterior portion of an embryo a few hours before hatching time. About 160 hours. × 60.

FIG. 3. The base of the thalamencephalon, the foregut, and the stomodaeum of the same section under stronger magnification. × 190.

FIG. 4. Median sagittal section through the anterior portion of a larva soon after hatching, early in the eighth day. × 60.

FIG. 5. The roof of the mouth and the hypophysis of the same section more highly magnified. × 190.

FIG. 6. Transverse section through the brain and hypophysis of a larva about eight days old. × 60.

FIG. 7. Median sagittal section through the hypophysis and base of the thalamencephalon of a larva nearly nine days old. × 190.

FIG. 8. Transverse section through the hypophysis and base of the infundibulum of a larva during the tenth day. × 190.

FIG. 9. Median sagittal section through the hypophysis and infundibulum of a larva ten days old. × 60.

FIG. 10. Transverse section of the hypophysis and base of the infundibulum of a larva ten days old. × 190.

FIG. 11. Median sagittal section through the hypophysis and infundibulum of a larva fourteen days old. × 60.

FIG. 12. Median sagittal section through the hypophysis and adjacent parts of a larva about the same age as the preceding. × 190.

FIG. 13. Transverse section of the hypophysis and infundibulum of a larva about fifteen days old. × 60.

FIG. 14. Transverse section through the hypophysis and infundibulum of a larva between twenty-two and twenty-six days old. × 60.

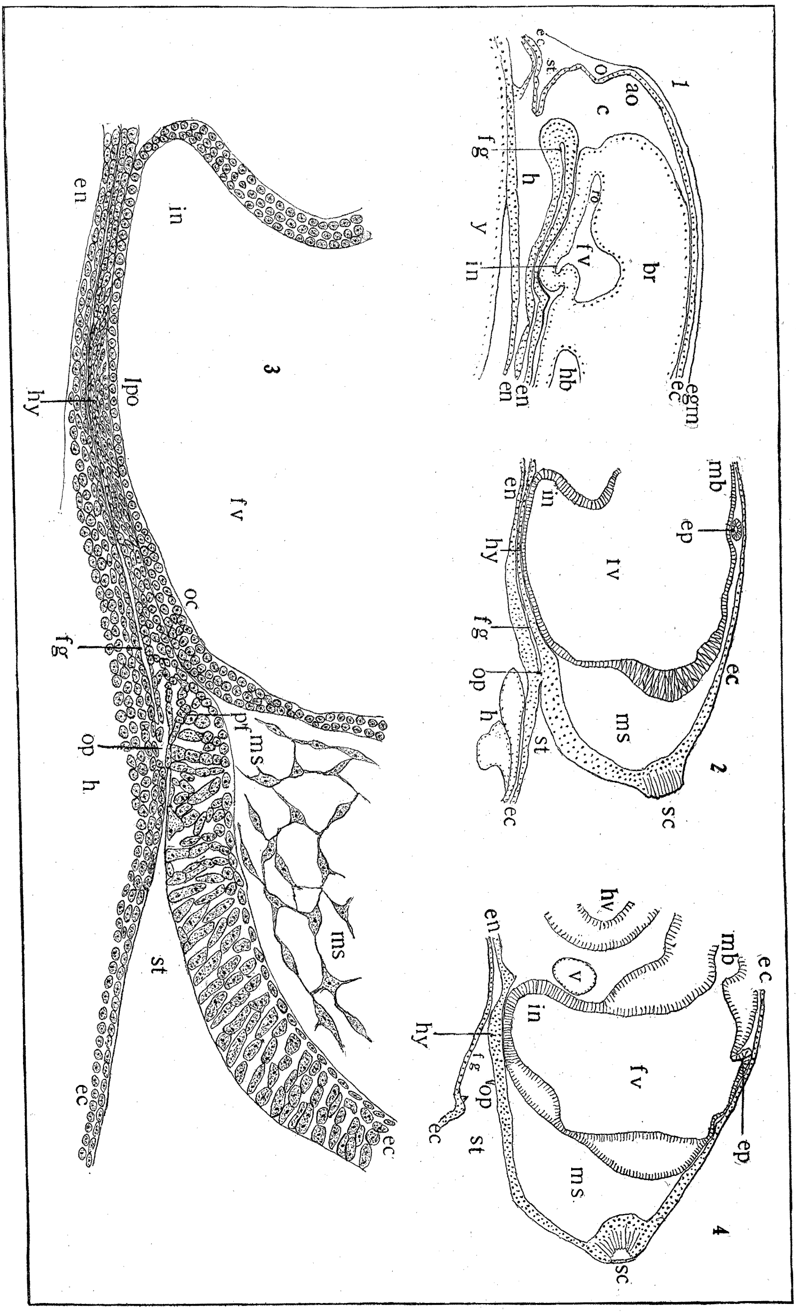
FIG. 15. The hypophysis of the same section more highly magnified. × 215.

FIG. 16. Median sagittal section through the hypophysis and infundibulum of a larva between thirty and thirty-five days old. × 60.

FIG. 17. Transverse section through the hypophysis and infundibulum of a larva of the same age as the preceding. × 60.

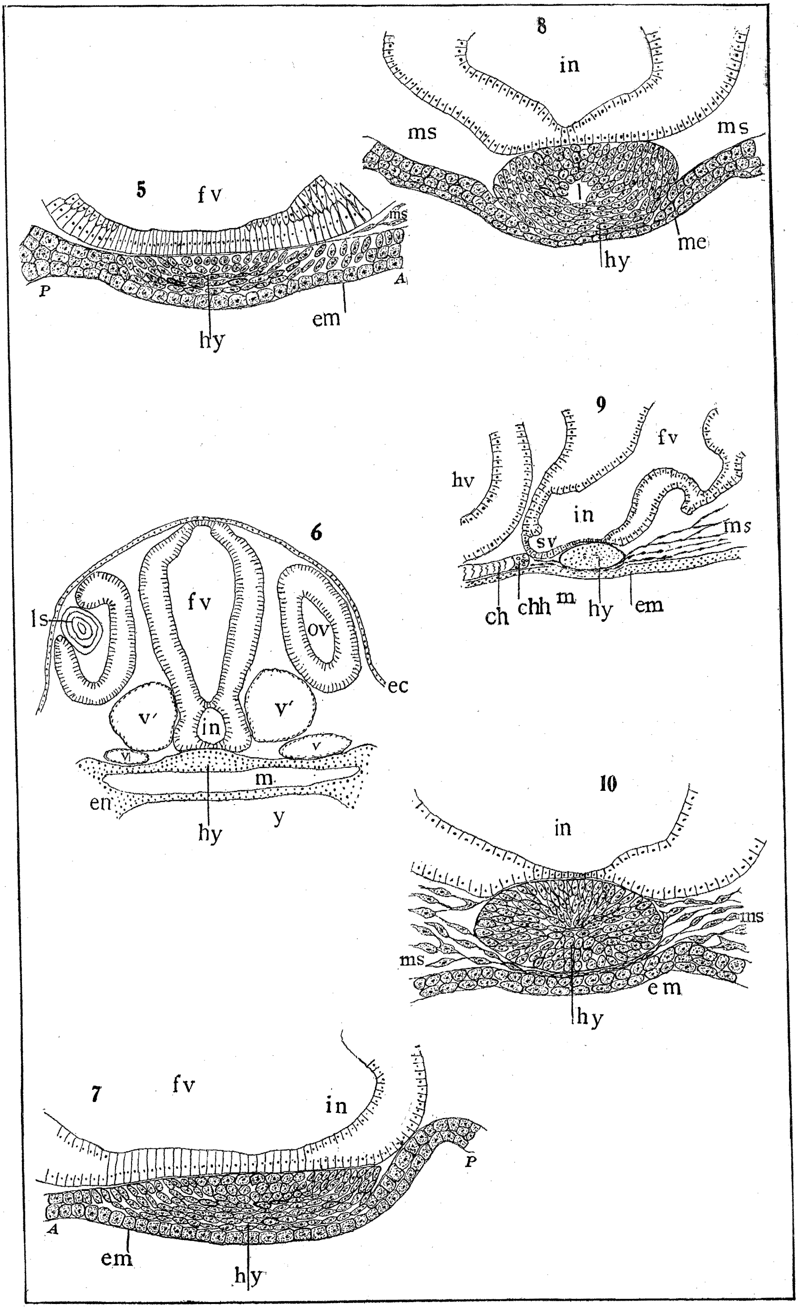
FIG. 18. A nearly horizontal section through the hypophysis of a larva about thirty days old. × 105.

Plate I.



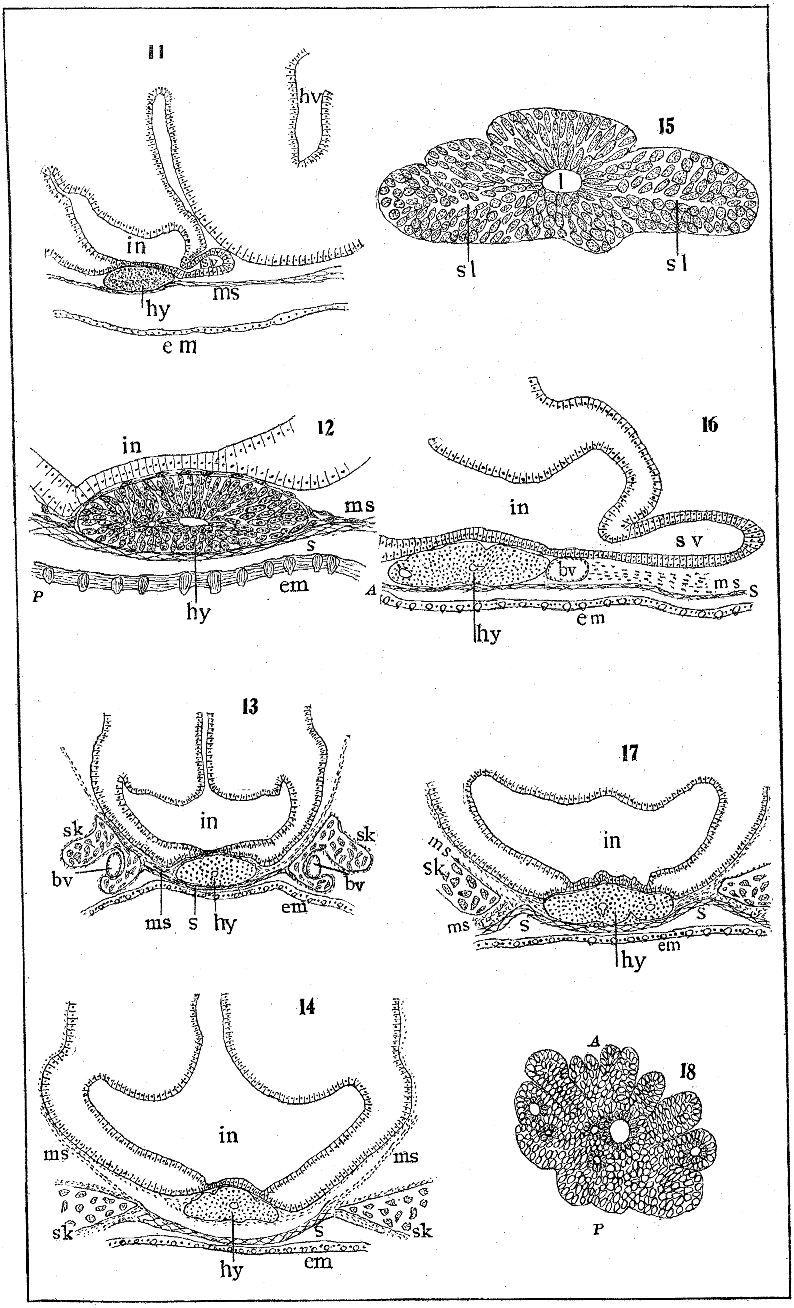
J. M. P. del.

Plate II.



J. M. P. del.

Plate III.



J. M. P. del.